This report is required by law (7 USC 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 2150.

See reverse side for additional information.

Interagency Report Control No 0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

ANNUAL REPORT OF RESEARCH FACILITY

(TYPE OR PRINT)

1. REGISTRATION NO. 23-R-0016 **CUSTOMER NO.** 289

FORM APPROVED
OMB NO. 0579-0036

2. HEADQUARTERS RESEARCH FACILITY *(Name and Address, as registered with USDA, include Zip Code)*

UNIVERSITY OF PITTSBURGH
3500 TERRACE STREET S1040 BIO SCI. TWR.
PITTSBURGH, PA 15261

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, teaching, or experimentation, or held for these purposes. Attach additional sheets if necessary.)

FACILITY LO(

(b)(2)High, (b)(7)(F)

(b)(2)High, (b)(7)(F)

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY *(Attach additional sheets if necessary or use APHIS FORM 7023A)*

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic,analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. *(An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used must be attached to this report)*	F. TOTAL NO. OF ANIMALS (Cols. C + D + E)
4. Dogs			101		101
5. Cats	1		66	19	85
6. Guinea Pigs			12		12
7. Hamsters	116	63	201		264
8. Rabbits	8	241	1016		1257
9. Non-Human Primates	49	14	699	28	741
10. Sheep			35		35
11. Pigs		28	408		436
12. Other Farm Animals					
Calves			21		21
13. Other Animals					
Prairie Dogs			6		6
Ferrets			96		96

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). **A summary of all the exceptions is attached to this annual report.** In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer or Legally Responsible Institutional official)
I certify that the above is true, correct, and complete (7 U.S.C. Section 2143)

SIGNATURE OF C.E.O. OR INSTITUTIONAL OFFICIAL	NAME & TITLE OF C.E.O. OR INSTITUTIONAL OFFICIAL *(Type or Print)*	DATE SIGNED
Provided in hard copy	(b)(6),(b)(7)(c)	11/28/2007

APHIS FORM 7023 (AUG 91) **(Replaces VS FORM 18-23 (Oct 88), which is obsolete**

PART 1 - HEADQUARTERS

This report is required by law (7 USC 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 2150.

See reverse side for additional information.

Interagency Report Control No 0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

1. REGISTRATION NO. 23-R-0016 **CUSTOMER NO.** 289

FORM APPROVED
OMB NO. 0579-0036

CONTINUATION SHEET FOR ANNUAL REPORT OF RESEARCH FACILITY

(TYPE OR PRINT)

2. HEADQUARTERS RESEARCH FACILITY *(Name and Address, as registered with USDA, include Zip Code)*

UNIVERSITY OF PITTSBURGH
3500 TERRACE STREET S1040 BIO SCI. TWR.
PITTSBURGH, PA 15261

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY *(Attach additional sheets if necessary or use this form.)*

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic,analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. *(An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used must be attached to this report)*	F. TOTAL NO. OF ANIMALS **(Cols. C + D + E)**
Goats	3		35		35

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). **A summary of all the exceptions is attached to this annual report.** In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer or Legally Responsible Institutional official)
I certify that the above is true, correct, and complete (7 U.S.C. Section 2143)

SIGNATURE OF C.E.O. OR INSTITUTIONAL OFFICIAL	NAME & TITLE OF C.E.O. OR INSTITUTIONAL OFFICIAL *(Type or Print)*	DATE SIGNED
Provided in hard copy	(b)(6),(b)(7)(c)	11/28/2007

APHIS FORM 7023A **(AUG 91)** **(Replaces VS FORM 18-23 (Oct 88), which is obsolete**

PART 1 - HEADQUARTERS

APHIS Form 7023 Column E Explanation

This form is intended as an aid to completing the APHIS Form 7023 Column E explanation. It is not an official form and its use is voluntary. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

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1. Registration Number: 23-R-0016

2/3. Species (common name) & Number of animals used in this study:

Non-Human Primates (28)

4. Explain the procedure producing pain and/or distress.

Below is all explanation for NHPs.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

See above.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency: CFR:

APHIS Form 7023 Column E Explanation

This form is intended as an aid to completing the APHIS Form 7023 Column E explanation. It is not an official form and its use is voluntary. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

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1. Registration Number: 23-R-0016

2/3. Species (common name) & Number of animals used in this study:

Cats (19)

4. Explain the procedure producing pain and/or distress.

Below is all explanation for cats.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

See above.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency: CFR:

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Summary of exceptions to the regulations and standards, specified and explained by the principal investigators and approved by the IACUC

IACUC review and justification for exception to AWA Regulation: CFR Title 9 1.A to section 3.6 for resting surfaces. Four protocols from two investigators were approved for the removal of mandated resting boards for cats.

Protocols: 0508947, 0607291, 0610768 and 0705681

Species affected: Cats

Dispensation from the use of resting board in feline caging systems

Explanation: The resting surface required for cats under the AWA was removed after vestibular system lesions. Animals become posturally unstable following these lesions, such they may injure themselves when trying to jump onto the raised platform. In addition, this metal structure is approximately at the level of the animal's head, and damage to head implants could occur if the animal stumbles into the platform as a result of its postural stability.

A mat is placed on the bottom of the cage to provide a comfortable resting surface after the raised metal platform is removed.

Category E Explanations 23-R-0016 2007

Certificate Number: 23-R-0016; 289
University of Pittsburgh
USDA Annual Report for the Reporting Period 10/1/2006 through 9/30/2007

Column E Explanations

1) Registration Number: 23-R-0016
2) Species (common name) used in study: Cat
3) Number of animals used in this study: 1
4) Explanation of procedures producing pain and/or distress and scientific justification why pain and/or distress could not be relieved: Protocol # 0405038 Justification from Protocol/ PI: Spinalization will be performed under isoflurane anesthesia. Ketoprofen (2-3 mg/kg, 3 days) will be given on the days following spinalization. Additional analgesics will be given according to the DLAR veterinarian's recommendations. Currently there is no appropriate treatment to further alleviate any possible distress due to the animal's inability to move their hind limbs; therefore they are classified as category E.

Category E Explanations 23-R-0016 2007

1) Registration Number: 23-R-0016
2) Species (common name) used in study: Cat
3) Number of animals used in this study: 3
4) Explanation of procedures producing pain and/or distress and scientific justification why pain and/or distress could not be relieved: Protocol # 0609643 Justification from Protocol/ PI: Spinalization will be performed under isoflurane anesthesia. Ketoprofen (2-3 mg/kg, 3 days) will be given on the days following spinalization. Additional analgesics will be given according to the DLAR veterinarian's recommendations. Currently there is no appropriate treatment to further alleviate any possible distress due to the animal's inability to move their hind limbs; therefore they are classified as category E.

Category E Explanations 23-R-0016 2007

1) Registration Number: 23-R-0016
2) Species (common name) used in study: Cat
3) Number of animals used in this study: 2
4) Explanation of procedures producing pain and/or distress and scientific justification why pain and/or distress could not be relieved: Protocol #: 0508947 Justification from Protocol/ PI: The goal of this study was to determine the role of the vestibular system in adjusting the movement of air into and out of the lungs. A number of studies have demonstrated that the vestibular system contributes to regulating respiratory muscle activity, and this experiment was conducted to ascertain the physiological role of the responses. The experimental approach was to record the pressure, volume, and velocity of airflow into and out of the lungs of conscious animals adapted to lying on a tilt platform, both before and after the surgical elimination of vestibular inputs (through a bilateral labyrinthectomy). Bilateral labyrinthectomies render the animal posturally unstable, which is potentially distressful. Anesthetics were employed during every surgery, and analgesia was delivered after every surgery. Nonetheless, deep sedation would be required so assure that animals were not distressed by the postural instability and balance deficits that they experienced immediately following removal of vestibular inputs. Such a level of analgesia would not be prudent because it would impact on the data collected after the surgery and would also interfere with the animal's compensation for the effects of the lesion. It is well established in the human literature that compensation after vestibular lesions occurs more readily if movement is attempted than if the patient remains sedentary. Vestibular rehabilitation is based on the notion that improvement can only occur following vestibular lesions if subjects make frequent head and body movements. Thus, even if we were to sedate animals for several days following surgery, they would likely have experienced distress after the sedation is discontinued (as they did not compensate for the lesion after surgery). We thus deemed it most beneficial both scientifically and for the long-term condition of the animal to refrain from providing sedation following removal of vestibular inputs, although this may have resulted in a short period of distress before the animal adapted to its balance disorder.

Category E Explanations 23-R-0016 2007

1) Registration Number: 23-R-0016
2) Species (common name) used in study: Cat
3) Number of animals used in this study: 10
4) Explanation of procedures producing pain and/or distress and scientific justification why pain and/or distress could not be relieved: Protocol #: 0610768 Justification from Protocol/ PI: The goal of this study was to map the descending pathways from the brain that regulate muscle blood flow. The study was performed by injecting the transneuronal tracer rabies virus into a hind limb muscle. To accomplish our objectives, a spinal transection at the L2 level was needed, because rabies virus is transported to the nervous system via both motor neurons and sympathetic nervous system neurons innervating muscles. By transecting the spinal cord anterior to the hind limb motor neurons but posterior to the sympathetic preganglionic neurons innervating the muscle injected with rabies, the descending pathways providing inputs to motor neurons were prevented from becoming infected with virus. Anesthetics were employed during every surgery, and analgesia was provided during the postsurgical period. This protocol was deemed to be Category E only because of the possible distress resulting from lower body paralysis. Drugs or other means cannot easily eliminate such distress related to a debilitating condition.

Category E Explanations 23-R-0016 2007

1) Registration Number: 23-R-0016
2) Species (common name) used in study: Cat
3) Number of animals used in this study: 3
4) Explanation of procedures producing pain and/or distress and scientific justification why pain and/or distress could not be relieved: Protocol #: 0607291 Justification from Protocol/ PI: The goal of this study was to determine the consequences of removal of vestibular inputs on the activity of neurons in the vestibular nuclei, the brainstem structures that receive inputs from the vestibular portion of the inner ear. For this purpose, it was necessary to record activity from vestibular nucleus neurons before and after the bilateral surgical removal of vestibular inputs (bilateral labyrinthectomy). Bilateral labyrinthectomies render the animal posturally unstable, which is potentially distressful. Anesthetics were employed during every surgery, and analgesia was delivered after every surgery. Nonetheless, deep sedation would be required so assure that animals were not distressed by the postural instability and balance deficits that they experienced immediately following removal of vestibular inputs. Such a level of analgesia would not be prudent because it would impact on the data collected after the surgery and would also interfere with the animal's compensation for the effects of the lesion. It is well established in the human literature that compensation after vestibular lesions occurs more readily if movement is attempted than if the patient remains sedentary. Vestibular rehabilitation is based on the notion that improvement can only occur following vestibular lesions if subjects make frequent head and body movements. Thus, even if we were to sedate animals for several days following surgery, they would likely have experienced distress after the sedation is discontinued (as they did not compensate for the lesion after surgery). We thus deemed it most beneficial both scientifically and for the long-term condition of the animal to refrain from providing sedation following removal of vestibular inputs, although this may have resulted in a short period of distress before the animal adapted to its balance disorder.

Category E Explanations 23-R-0016 2007

1) Registration Number: 23-R-0016
2) Species (common name) used in study: Cebus apella
3) Number of animals used in this study: 1
4) Explanation of procedures producing pain and/or distress and scientific justification why pain and/or distress could not be relieved: Protocol # 0604760A-3 Justification from Protocol/ PI: All the animals used in Experiment #1 (n=5; see section 67) belong in Category E. The Category E classification is indicated because the injection of herpes simplex virus type I (HSV1) into the spinal cord of these animals is expected to cause significant morbidity that cannot entirely be relieved with analgesic or tranquilizing agents. Briefly, under anesthesia and aseptic conditions, we perform a craniotomy on these animals for the purpose of injecting a conventional retrograde tracer into M1 or the PMv. This surgery is not expected to cause postoperative morbidity that cannot be relieved with conventional analgesics. After a 10-14 day period that allows for both the transport of the neuronal tracer and the post-operative recovery of the animal, the animal will undergo a second surgery. In this aseptic surgery, the anesthetized animal will undergo a laminectomy and the injection of the H129 strain of HSV1 into the cervical spinal cord. The animal will require a 4 day post-surgical period before euthanasia. It is during this 4 day period after the 2nd procedure that we anticipate a progressive paresis eventually involving all four extremities (beginning during the third day), as well as the potential for a neuropathic pain state (beginning on the first or second day; refer to Section 63 above). Whereas we will provide pharmacological therapies for pain relief (see Section 63), we cannot prevent or reverse the paresis. Although we plan on the use of lorazepam as an anxiolytic during this second post-surgical survival period, we cannot be certain whether this pharmacological agent will entirely relieve the degree of distress that the animal will experience as it finds itself unable to move its extremities. We believe that our animals should be classified in Category E because it is our expectation that the animals will develop an irreversible, limb paresis after receiving HSV1 injections into the spinal cord. We expect this to occur on the third day of the four day survival period. While we can provide supportive care and pharmacological therapy for the animals (see above), we cannot fully know the degree of psychological distress that the animals will face in such a debilitated state despite our efforts for treatment. Therefore, because the paresis will be irreversible, it is considered a permanent, physiological impairment and the animal can be regarded as psychologically distressed until the end of the survival period is reached. It is for this reason that the animals that will receive HSV1 injections into the spinal cord have been listed in Pain Classification E. At no time will they be denied full access to pharmacological agents that will strive to minimize the animals' pain or distress.

1) Registration Number: 23-R-0016

2) Species (common name) used in study: M. Fascicularis (cyno)

3) Number of animals used in this study: 12

4) Explanation of procedures producing pain and/or distress and scientific justification why pain and/or distress could not be relieved:

Protocol # 0505270

Justification from Protocol/ PI: (type or copy here):

Bacillus anthracis, the causative agent of anthrax, is a major bioterriorist threat. As such, it is considered a Category A agent by the NIH and CDC. A complete understanding of how *Bacillus anthracis* kills its human host is essential to the development of vaccines and therapeutics aimed at prevention and control in the event of a bioterriorist attack. Although some information is available from nonhuman primate studies where animals have been exposed to *B. anthracis* spores, there is no information about the specific *in vivo* pathogenic effects of the two major toxins, Lethal toxin and Edema toxin, in any primate system. This study will determine for the first time the specific events associated with inoculation of macaques with purified toxin at the molecular level.

Although *in vitro* studies with human cells and *in vivo* studies in mice have identified several candidate virulence genes encoded by *Bacillus anthracis,* much remains to be learned about the nature and timing of these pathogenic events *in vivo.* Nonhuman primates are essential to this research because vaccines/therapies for the treatment/prevention of human disease are the ultimate goal of this research. The close phylogenetic relationship shared by old world monkeys and humans permits accurate predictions of the outcome of vaccine immunizations and treatments.

Old World monkeys and humans share a close phylogenetic relationship. These similarities allow easy translation of the pathogenic outcome of experimentally inoculated monkeys to naturally exposed humans.

There is one report in the literature that describes the outcome of exposure of cynomolgus macaques to intact anthrax spores (Vasconcelos, et al, 2003). In this study, significant variation was observed among the animals tested. If we assume that a similar variation in response to purified toxin will be observed, then more than one animal per group must be used to accurately determine the overall response to toxin. In our past experiences in SIV-related studies, 5 animals per group has provided maximal variation in the tissue-specific responses to infection.

ADDENDUM: Monkeys will be monitored until they show signs of illness associated with toxemia so that tissues from moribund animals can be analyzed. Since there is a considerable variation in the time to death following toxic exposure among animals, it is imperative that the animal display signs before he is euthanized. Anesthetics, analgesics, sedatives, and/or tranquilizers will mask these signs in treated animals. We will, however, visually inspect these animals every 2 hours so that they can be euthanized as soon as these signs are apparent and thus avoid death as an endpoint.

N 2 5 2008

1) Registration Number: 23-R-0016

2) Species (common name) used in study:
m. fascicularis (cyno)

3) Number of animals used in this study: 15

4) Explanation of procedures producing pain and/or distress and scientific justification why pain and/or distress could not be relieved:

Protocol # 0704462

Justification from Protocol/ PI: (type or copy here):

Bacillus anthracis, the causative agent of anthrax, is a major bioterriorist threat. As such, it is considered a Category A agent by the NIH and CDC. A complete understanding of how *Bacillus anthracis* kills its human host is essential to the development of vaccines and therapeutics aimed at prevention and control in the event of a bioterriorist attack. Although some information is available from nonhuman primate studies where animals have been exposed to *B. anthracis* spores, there is no information about the specific *in vivo* pathogenic effects of the two major toxins, Lethal toxin and Edema toxin, in any primate system. This study will determine for the first time the specific events associated with inoculation of macaques with purified toxin at the molecular level.

Although *in vitro* studies with human cells and *in vivo* studies in mice have identified several candidate virulence genes encoded by *Bacillus anthracis,* much remains to be learned about the nature and timing of these pathogenic events *in vivo.* Nonhuman primates are essential to this research because vaccines/therapies for the treatment/prevention of human disease are the ultimate goal of this research. The close phylogenetic relationship shared by old world monkeys and humans permits accurate predictions of the outcome of vaccine immunizations and treatments.

Old World monkeys and humans share a close phylogenetic relationship. These similarities allow easy translation of the pathogenic outcome of experimentally inoculated monkeys to naturally exposed humans.

There is one report in the literature that describes the outcome of exposure of cynomolgus macaques to intact anthrax spores (Vasconcelos, et al, 2003). In this study, significant variation was observed among the animals tested. If we assume that a similar variation in response to purified toxin will be observed, then more than one animal per group must be used to accurately determine the overall response to toxin. In our past experiences in SIV-related studies, 5 animals per group has provided maximal variation in the tissue-specific responses to infection.

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